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THE 3D STRUCTURE OF SOME DIARRHEAL CAUSING BACTERIAL TOXINS

ANNUAL REPORT

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The 3D maps of two (2) crystallography forms of SEB have been prepared to 3A resolutions. Analyses of these maps is in progress. The two (2) crystollography forms are strickenly similar in molecular structure, as expected.

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FOREWORD

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INTRODUCTION:

The urgency to know the 3D structure of SEB is intensifying since there is a widespread effort currently to understand the mechanisms of its biological actions and of those of related staphylococcal enterotoxins (SEs), especially its toxicity and its extraordinary immunogenic activity, i.e., its superantigenicity (1,2) With respect to its role as a superantigen, certain amino acid residues in SEB have been demonstrated to be essential for interaction with the V beta T cell receptor while others have been shown to be crucial for binding with the MCH-II molecule (3), but the spatial relationships of these important functional residues reain unknown. The information will be evident in the 3D structure. The residues which target the V betaa receptor are thought to be located close to each other on the protein surface although they occur in different parts of the amino acid sequence (3). The same investigators find that the MHC binding regions of SEB lie in the interior of the molecule. The protein accordingly is believed to undergo a change its conformation before it is able to bind to the MHC-II molecule. The sequential bindings of the two different receptors constitute essential steps in the "superantigenicity" process. In SEB the superantigenic activity resides in the domain which contains the amino plus of the protein (3). SEB is composed two domains, and it is believed that the amino containing protions is the larger of the two (3).

Other important biological and biochemical properties of the SEs have been correlated with the protein sequences. Again definitive knowledge of the 3D structure of SEB is needed to fully understand the stereochemical basis underlying those properties.

BODY:

During the past year we were able to trace approximately 80% of the main chain in SEB. Our efforts to correlate the 3A electron density maps and the amino acid sequence proved elusive. Extending the phases to higher resolution yielded no additional information. Attention was shifted to improving the quality of the x-ray diffraction data. We discovered a 3rd crystal form of SEB which diffracted to 2.1A and gave good quality derivative data. We collected x-ray data on the synchrotron at the Brookhaven National Laboratory with the aim of measuring the anomalous dispersion signal for use in phasing. However the new experimental data yielded an electron density map which was comparable in quality to previous ones. Actually the electron density maps of the two forms showed only very minor differences in molecular structure. We next focused our attention on fitting three dimensional models to the 3A map. Many different models were tested, but none refined successfully. At this resolution we could not identify the single disulfide linkage in the map, but we came up with 3 stereochemically acceptable possibilities. Our initial model was based on the assumption that we could clearly identify one of the chain termini. We looked for recognizable features in the map which were or might be related to the sequence. We found a region of strong side chain density which approved to accomodate F196, W197, and Y198.

While we were testing the various structural models, it was clear to us that the difficulty in correlating the side chains and the sequence along with the trouble in tracing the main chain stemmed from the high percentage of large side chains in the molecule and from the large number of possibilities to form salt bridges. However, all of the maps contained characteristic helices and beta sheet regions. We, therefore, turned our attention to identifying where the helical regions occurred in the sequence. We came up with three regions and a possible fourth. We then attempted to trace the main chain on the assumption that we knew the position and orientation of one of the putative helical regions. Encouragingly this put the S-S at one of the potential locations previously identified as a disulfide. However in order to make the main chain segments agree with the sequence, we changed parts of the main trace arbitrarily. Encourangingly these changes placed the four helical segments in the model at positions in the main chain which agreed very well with our predictions of where they ought to occur in the sequence. Currently we are testing this model.

CONCLUSIONS:

Tentatively speaking we temporarily conclude the following about the 3D structure of SEB:

1. It consists of 2 domains of slightly different sizes.

2. There are at least 3 helices and possibly four ranging from two to four turns in length.

3. There are several beta sheet regions.

4. There seem to be several salt bridges but at 3A and in the absence of a refinable model, this interpretation of these features is speculative.

REFERENCES:

1. Marrack, P. T Kappler, J. Science 248, 705-711 (1990).

2. Spero et al. In Bacterial Toxins, Vl 4, Ed. C Hardegree and A. Tu, Marcel Dekker, Inc., New York, NY (1998).

3. Kappler et al. Personal Communication (1991).